

CLAIMS

What is claimed is:

- 5 1. A method for detecting a difference in the sequence of two nucleic acid molecules comprising:
 - a. Contacting said two nucleic acids under conditions that allow the formation of a four-way complex and branch migration;
 - 10 b. Contacting said four-way complex with a tracer molecule and a detection molecule under conditions in which the detection molecule is capable of binding the tracer molecule or the four-way complex; and
 - c. Determining binding of tracer molecule to the detection molecule before and after exposure to said four-way complex, wherein reduced binding after contact indicates the presence of a difference between the two nucleic acids.
- 15 2. A method for detecting a difference in the sequence of two nucleic acid molecules comprising:
 - a. Contacting said two nucleic acids under conditions that allow the formation of a four-way complex and branch migration;
 - 20 b. Contacting said four-way complex with a tracer molecule and a detection molecule under conditions in which the detection molecule is capable of binding the tracer molecule or the four-way complex; and
 - c. Comparing binding of said tracer molecule said detection molecule in b. with binding of said tracer molecule to said detection molecule in said test solution without said four-way complex, wherein reduced binding in b. indicates a difference between the two nucleic acids.
- 25 3. The method of Claim 1 or 2, wherein steps a. and b. are carried out simultaneously.
- 30 4. The method of Claim 1 or 2, wherein under said branch migration conditions said four-way complex is capable of resolution if the nucleic acids are identical in sequence.
5. The method of Claim 1 or 2, wherein under said branch migration conditions said four-way complex is not capable of resolution if the nucleic acids are not identical in sequence.
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6. The method of Claim 1 or 2, wherein under said branch migration conditions if a difference between said two related nucleic acid sequences is present, branch migration in said four-way complex ceases and said four-way complex is stabilized, and if no difference between said two related nucleic acid sequences is present, branch migration in said
5 four-way complex continues until complete strand exchange occurs and said four-way complex resolves into two duplex nucleic acids, thereby forming a stabilized four-way complex.

7. The method of Claim 1 or 2, wherein the difference is a mutation, an insertion, a
10 deletion or a single base substitution.

8. The method of Claim 1 or 2, wherein one of the nucleic acids is DNA.

9. The method of Claim 1 or 2, wherein said four-way complex comprises a Holliday
15 junction.

10. The method of Claim 1 or 2, wherein said detection molecule is capable of selectively binding a four-way nucleic acid complex.

20 11. The method of Claim 10, wherein said detection molecule is capable of selectively binding a Holliday junction.

12. The method of Claim 11 wherein said detection molecule is selected from the group consisting of RuvA, RuvC, RuvB, RuvA, RuvG, Cce1, spCce1, Hjc and mutants or analogs
25 thereof.

13. The method of Claim 11, wherein said detection molecule is thermostable.

14. The method of Claim 1 or 2, wherein said tracer molecule is a nucleic acid
30 comprising a stable four-way complex.

15. The method of Claim 1 or 2, wherein said tracer molecule is a nucleic acid comprising an immobile four-way complex.

35 16. The method of Claim 14, wherein said tracer molecule comprises one, two, three or

four oligonucleotides.

17. The method of Claim 1 or 2, wherein said tracer molecule is capable of selectively binding the detection molecule.

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18. The method of Claim 1 or 2, wherein said tracer molecule comprises a detectable label.

19. The method of Claim 18, wherein said detectable label is capable of generating a signal upon binding of said tracer molecule to said detection molecule.

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20. The method of Claim 18, wherein said detectable label is a fluorescent label.

21. The method of Claim 20, wherein said fluorescent label is selected from the group consisting of fluorescein, rhodamine, cy dyes or BODIPY.

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22. A tracer molecule comprising a detectable label and a nucleic acid complex, wherein said nucleic acid complex comprises a stable four-way junction.

23. The tracer molecule of Claim 22, wherein said tracer molecule comprises one, two three or four oligonucleotides.

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24. The tracer molecule of Claim 22, wherein said tracer molecule is capable of generating a signal upon binding to a detection molecule.

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25. The tracer molecule of Claim 22, wherein said detectable label is a fluorescent label.

26. The tracer molecule of Claim 22, wherein said fluorescent label is selected from the group consisting of fluorescein, rhodamine, cy dyes and BODIPY.

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